

**CYANOTOXINS EXPOSURE AND ATTRIBUTABLE HEALTH EFFECTS
AMONG RESIDENTS OF THARAKA-NITHI COUNTY, KENYA**

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REG NO: Q139/CE/24640/2012

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF
SCIENCE (ENVIRONMENTAL HEALTH) IN THE SCHOOL OF HEALTH
SCIENCES OF KENYATTA UNIVERSITY.**

NOVEMBER 2022

DECLARATION

This research thesis is my original work and has not been presented for a degree award in any other University or for any other award.


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DEDICATION

This thesis is dedicated to my Almighty God for His grace that has enabled me to go this far, not forgetting my wife, my children, my parents, my sister, and my brothers for their support and encouragement.

ACKNOWLEDGMENT

I acknowledge my supervisors, Dr. Daniel Akunga and Dr. Peterson Warutere for their wise counsel, guidance, correction, encouragement and support.

I acknowledge the National Commission for Science, Technology and Innovation for financial support, Kenya Water Institute, Kenya National Museum and Government Chemist for assistance in sample collection and analysis. Special thanks go to Tharaka Nithi County, Tharaka South Sub County Public Health, household heads of the sampled households and research assistants for their participation in the study. God bless you all.

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DEFINITION OF KEY TERMS

Anatoxin a: A bicyclic secondary amine alkaloids neurotoxin produced by cyanobacteria

Composite Sample: A sample composed of several grab samples which are then combined to form one sample

Cyanobacteria: Blue-green algae or Cyanophyta, phylum of photosynthetic bacteria

Cyanotoxins: Secondary metabolites toxins released by cyanobacteria cells

Cylindrospermopsin: A tricyclic alkaloid toxin produced by strains of various species of cyanobacteria with neuro-, cyto-, dermato-, hepato-toxicities

Cyanotoxin related health problems: Disease symptoms attributed to cyanotoxins

Hepatotoxins: Cyanotoxins affecting the liver

Household heads: A father or mother who is in-charge of a homestead or any dependable household member above 18 years of age.

Microcystins: naturally occurring cyclic peptides hepatotoxins produced by strains of various species of cyanobacteria. Can also affect the kidney and reproductive system

Neurotoxins: Cyanotoxin that affecting nervous

Quality Assurance: an integrated system of management activities involving planning, implementation, documentation, assessment, reporting, and quality improvement to meet what is expected

Turbidity. Measure of the cloudiness of water due to dissolved or suspended particles

ABBREVIATIONS AND ACRONYMS

CyanoHABs	Cyanobacteria Harmful blooms
Cyanot	Cyanotoxins
DMB	Drought Monitoring Bulletin
EPA	Environmental Protection Agency
ER	Eye Related symptoms
GoK	Government of Kenya
GR	Gastrointestinal Related
GSS	Ghana Statistical Service
JP	Joint Pain
nd	Not detected
NTU	Nephelometric Turbidity Units
NV	Nausea and Vomiting
PDA	Photo Diode Array
SR	Skin Related
T	Temperature
TN	Total nitrogen
TNCIDP	Tharaka Nithi County Integrated Development Plan
TP	Total Phosphorous
TR	Throat Related
Tur	Turbidity
USGS	United States Geological Survey

ABSTRACT

Access to safe domestic water remains a challenge in many rural community and water scarce areas. One key challenge is cyanotoxins in surface water that has been known to cause serious health effects among people using these sources. The main objective of the study was to determine health effects attributable to cyanotoxin exposure among residents of Tharaka-Nithi County. The study employed Analytical cross-sectional study design in which quantitative data was collected. Interview schedules were main subject ways of data collection from the household heads while water samples for cyanotoxins and physicochemical characteristics were subjected to laboratory analysis. Simple random sampling was used to select 6 water sources. Treated pipe water and residents using it were the control point. The number of households sampled was proportionate to the number of the households in the particular area which were subjected to systematic random sampling technique during data collection. Household heads or any other dependable adult present in the household at the time of interview and were willing to participate were illegible for participation. Those excluded in the study are those who met the inclusion criteria but were absent during the sampling or unwilling to participate. A household to start with was randomly picked and the calculated interval followed in a chosen direction until the required number of households was reached. Data was analyzed using SPSS version 21 and presented using tables and bar graphs. Majority (54.40%) of the respondents were of age 46 and above years. Majority (54.93%) of respondents had primary level of education. Majority (48.53%) of respondents earn less than KShs 10000 per month. Majority (36.59%) of the respondents with more than one disease symptoms related to cyanotoxins use water with high level of cyanotoxins ($3.6\mu\text{g/L}$). Two sources of water were found to have significant level of cyanotoxin ($3.6\mu\text{g/L}$ and $2.3\mu\text{g/L}$) above WHO recommended value ($1\mu\text{g/L}$). The physicochemical characteristics were found to be high in sources with detected cyanotoxins: -Total nitrogen (7.01mg/l); total phosphorous (2.16 mg/l); pH (9.8); turbidity (21.2 NTU) and temperature (27.6°C). Chi-square test showed that the level of income had significant influence on the selection of the water source while the age and level of education had no significant influence on selection of water source. Pearson correlation found that cyanotoxin levels in the water source influenced the disease symptoms related to cyanotoxins and physicochemical characteristics influenced the cyanotoxin levels in water source. The findings of this study exposed the situation on the ground on health effects related to cyanotoxins and cyanotoxins levels in water sources and hence a wake-up call to the Public Health Officer and other Sub-County authorities on safety of the water sources. It also alerts the residents on the level of cyanotoxins in water they use and effects of using such water sources.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Safe drinking water remains a challenge worldwide and approximates 2 billion people are at risk to contaminated water due to unimproved water sources commonly in illegal settlements and rural settings (WHO, 2022). Six percent of the world are exposed to unsafe water source which causes 1.2 million deaths per year (Ritchie and Roser 2021). These also exposes consumers to a number of waters related illnesses such as; diarrhea, arsenicosis, fluorosis, trachoma, scabies, schistosomiasis, malaria and dengue fever (WHO, 2019). Approximately 829,000 people die due to diarrhea (WHO, 2022). Microbial contamination occurs in all water sources such as piped water supplies, groundwater and surface water and can only be safe after treatment (Kremer *et al*, 2022). In low-income countries, one -in -four have no access to safe water which causes 6% of deaths (Ritchie and Roser 2021)

During rain seasons, the runoff from various places contaminates the surface water (Huisman *et al*, 2018). The storm water may contain the nutrient which may cause proliferation of HABs called cyanobacteria (Nienbar and Steinitz-Kannan, 2018). This is most common in swallow waters, in rural settings with high temperature and prolonged droughts followed by a period of rainfall (Huisman *et al*, 2018). The cyanobacteria have effects on disruptions of water supply, closure of recreation sites, unpleasant taste and odor (Svir̃cev *et al*, 2017). Cyanobacteria also produces cyanotoxins that causes ill health effects to animals and human being (Ho *et al*, 2019).

The three most prevalent cyanotoxins are: microcystin-LR, anatoxin-a, and cylindrospermopsin (WHO, 2017). Microcystins is the most common cyanotoxins

(EPA, 2019). Cyanotoxins are found in fresh water, marine water, and manmade water sources (EPA, 2017b). Animals, birds, and fish are poisoned when water has high levels of cyanotoxin (Pham and Utsumi, 2018). In human being, low level of 1 µg /L of cyanotoxins is unsafe (WHO, 2017). The symptoms caused by cyanobacterial toxins are; skin and eye irritation, skin rashes, vomiting, nausea, diarrhea, pneumonia, headache, liver damage, asthma, fever, blisters of the mouth, and muscle and joint pain (EPA, 2019). The toxins can enter animal or human body when they come into contact with the contaminated water or sea food (EPA, 2019). This occurs through ingestion, inhalation, or direct exposure to the skin. Drinking contaminated water with cyanotoxins or activities in water like recreation, swimming, playing sports or boating can expose a person to poisoning (WHO, 2020c).

Some parts of Tharaka Nithi County experiences periods of long droughts followed by periods of short rains forcing the residents from these regions to use water from dams (TNCIDP, 2017) which may be exposed to surface water contamination and may be unsafe for human use. Studies shows that most man-made waters are built in development lands with activities like cultivations which may be a source of nutrients (Doubek and Carey, 2017). Nutrient contamination can trigger cyanobacteria growth leading to cyanotoxin production (EPA, 2017b). The cyanotoxin levels determine whether the water is fit for domestic use (CRS, 2019). Where cyanotoxin are found in high levels in water source, there may be risks of cyanotoxin poisoning (Greer *et al*, 2018).

Lack of official state guidelines for cyanotoxin levels in drinking water has a great influence on risks to residents who use surface water which may be unsafe. This study

was therefore aimed at determining the cyanotoxin exposure and attributable health effects among residents of Tharaka-Nithi county. Level of cyanotoxins in water sources was compared with the World Health Organization (1µg/L) standard (WHO, 2017).

1.2 Problem Statement

Cyanotoxin in drinking water source has been known to cause serious health effects among people using these sources for their domestic water needs (Ho *et al*, 2019). Some of these effects include gastrointestinal, lethargy, dermatological, headache and fever (Roberts *et al*, 2020). They can also cause kidney and liver damage (WHO 2020c), and even lead to liver cancer and death (Fiore *et al*, 2020). The cost of global health burden was estimated at \$12,605 for digestive illness and \$14,600 for respiratory illness (Christian *et al*, 2019). Cyanobacteria in domestic surface water sources is usually a function of elevated warm temperature, sharing of water sources with animals (Wallace *et al*, 2016), soil erosion from agricultural field, eutrophication and climate change (Gilbert *etal*, 2020) mostly caused by human activities (Paerl, 2020). This have degraded water quality (Westrick *et al* 2018), causing foul smell and unaesthetic challenges to the environment (Huisman *et al*, 2018). This may cause closures of recreational sites (Svirčev *et al*, 2017); USA estimated cost of impact of over US\$ 2.2 billion per year (WHO, 2019).

It is therefore important that regular testing of domestic water sources for presence of cyanobacteria and associated toxins to protect human health(EPA, 2019). Residents of Tharaka-South Sub County largely depends on surface water for their domestic water source. Nevertheless, there is lack of documentation as to the level of cyanobacteria and

associated toxins in these water bodies so as to provide accurate advice to residents. Indicative health challenges recorded in Tharaka Nithi County Integrated Development Plan 2018-2022 suggest symptoms associated with cyanotoxin exposure as presented in the table below;

Health effects related to cyanotoxins	Number of people affected	Reference
Upper respiratory tract infections	133294	TNCIDP, 2017
Disease of Respiratory System	107381	TNCIDP, 2017
Disease of the skin	63738	TNCIDP, 2017
Joint pains	51771	TNCIDP, 2017

Table 1. 1 Health effects related to cyanotoxins in Tharaka Nithi County

It is therefore necessary that the level of cyanotoxins in different drinking water sources in Tharaka-Nithi County and the reported health challenges among the residents of Tharaka Nithi County be determined and hence the study.

1.3 Justification of the Study

In Kenya, there is scarcity of information on cyanotoxins levels in domestic water sources. This is also not different with Tharaka South Sub-County as cyanotoxin levels in domestic water is not established. Tharaka South Sub-County was purposively selected. It is a semi-arid region and hence during rainfall seasons, water is stored in water pans, earth-dams and rock catchment for the purpose of use during the dry seasons. This was a baseline study that was to seek information about the symptoms related to cyanotoxins and cyanotoxins levels in the water sources particularly that in water reservoirs. The cyanotoxins levels in the water sources were then to be compared

to the laid down standards by WHO to inform policy. Residents in this region use water with no information on cyanotoxin levels which may risk them to cyanotoxins poisoning. The study also determined the physicochemical characteristics that influences cyanobacteria proliferations and sociodemographic factors that influences the choice of water source by the residents. The study therefore meant to help in policy enrichment, boost health and safety at the household level and the environment at large and provide useful information to the Public Health department on safety of water sources particularly on cyanotoxins. This could also offer useful information to the County and the Nation at large on addressing the issue of cyanotoxins.

1.4 Research Questions

1. What health problems related to cyanotoxins poisoning as reported by the residents of Tharaka Nithi County?
2. What is the level of cyanotoxins in man-made water sources in Tharaka Nithi County?
3. What are physicochemical characteristics in man-made water sources in Tharaka Nithi County?
4. What is the influence of socio-demographic factors on choice of water source by residents of Tharaka Nithi County?

1.5 Main Objective

To determine cyanotoxin exposure and attributable health effects among residents of Tharaka-Nithi County, Kenya

1.6 Specific Objectives

1. To assess the cyanotoxin poisoning related health problems reported by the residents of Tharaka Nithi County

2. To determine the level of cyanotoxins in man-made water sources in Tharaka Nithi County
3. To determine physicochemical characteristics in man-made water sources in Tharaka Nithi County
4. To assess the influence of socio-demographic factors on choice of water source by residents of Tharaka Nithi County

1.7 Study Significance and Anticipated Output

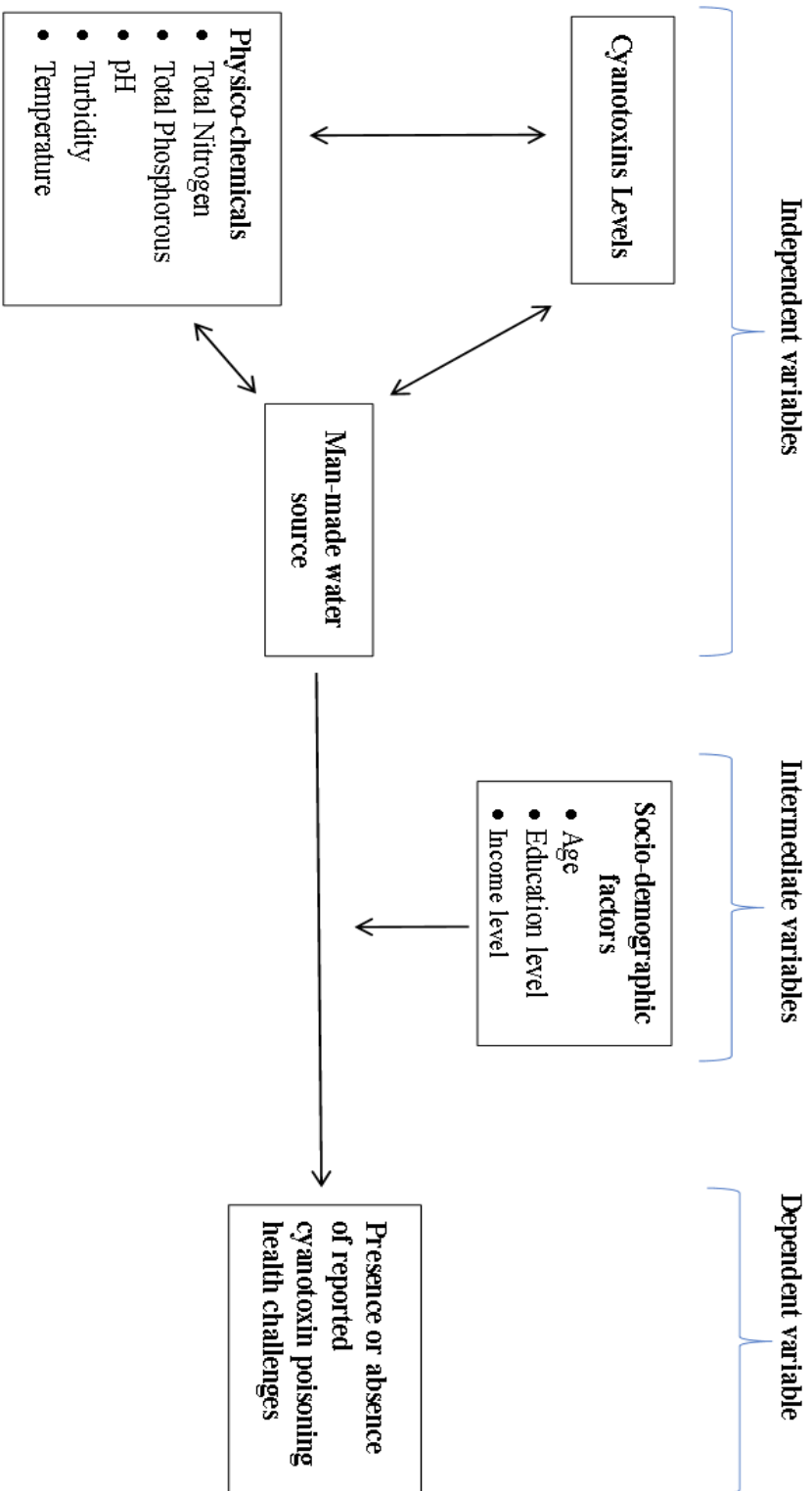
The study was to help create scientific information that will help in policy enrichment by providing baseline information on health effects associated to cyanotoxins and cyanotoxin levels in the selected water sources in Tharaka South Sub-County. It will also help Public Health Department, Ministry of Health (MOH), and local authorities by being a source of reference in strategizing ways of solving the problem of water safety. It will also empower the community by the members being sensitive on various adverse effects and potential hazards that may come with the choice of a water source for domestic use.

1.8 Limitation of the study

Tharaka South Subcounty is sparsely populated and water sources are located distance apart which required a lot of time in data collection, hence the motorcycles were used to overcome these challenges. The residents were also doubtful if they will be restricted to access water if it's not safe but they were assured of safety measures that could be used to improve the water quality if it's found unsafe and they will continue using the water. Majority of residences use Tharaka language hence research assistants were selected on basis of familiarity with the language

1.9 Conceptual Framework

The conceptual framework (Figure 1) illustrates the various inter-relationships between variables. The dependent variable was the presence or absence of reported cyanotoxin poisoning health challenges. The independent variables were the cyanotoxin levels, physicochemical characteristics and man-made water sources. The intermediate variables were socio-demographic factors



Adapted and modified from: H. Kenneth Hudnell, 2007

Figure 1 Conceptual Framework

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

The chapter reviews literature along four main themes. The first part was review on health effects related to cyanotoxins. The second part discussed on cyanotoxins levels. The third part was on the physicochemical characteristics that promote levels of cyanotoxins in water and last part was on sociodemographic factors that influences the choice of water sources for use.

2.2 Health effects related to cyanotoxins

Cyanobacteria releases cyanotoxins during cell death which can cause ill health effects when exposed to a person (EPA, 2019). Cases of human illnesses related to exposure to cyanotoxin are high (CDC, 2021). There are many types of cyanotoxins but the two main ones are classified as neurotoxins and hepatotoxins which affects specific organ (WHO, 2017). Examples of neurotoxin is anatoxin-a and hepatotoxins are cylindrospermopsin and microcystins (Roberts *et al.* 2020).

Neurotoxins affects the nervous system by inhibiting the control mechanisms of ions and communication between one neuron to another (WHO 2020a). Anatoxin-a poisoning has been reported in France where the infected people experienced symptoms as blurred eye sight, dizziness, double vision, tinnitus, impaired coordination and cramps in the legs and abdomen (Biré *et al.*, 2020).

Hepatotoxins cause damage to the liver cells (EPA, 2019). Cylindrospermopsin and microcystins can cause damage to liver as well as to the kidney (EPA, 2017b). Illness related to cylindrospermopsin poisoning was reported in Australia in 1980 where the patient who had consumed water from a source with cyanotoxin experienced symptoms as vomiting, liver enlargement and kidney problems (WHO, 2020b). Microcystins on

the other hand have been attributed to ill health effects in recreational and drinking water (Chorus and Welker, 2021). The ill health effects related to microcystins are gastrointestinal symptoms, eye irritations/reddening, dermal irritations/rashes, headaches, fever and kidney and liver damage (WHO, 2020c). Microcystin breakdown the liver cells causing the health effects (Chorus and Welker, 2021). Variability in factors affecting absorption and distribution of microcystins are poorly understood at present (EPA, 2015). Primary elimination occurs by the urinary and faecal routes while secondary elimination occurs through hair follicles (Foss *et al*, 2019)

In the year 2016 and 2018, CDC reported 389 cases related to cyanotoxins in United States at a lake in Utah where 67% of victims had gastrointestinal, 43% lethargy, 27% skin related, as well as headache and fever (Roberts *et al*. 2020). In Montevideo, Uruguay, a child of 20 months was exposed to microcystins through repeated recreational exposure to blooms containing microcystins (Vidal *et al*, 2010).

In Tharaka Nithi, access to clean water, for both animal and human, is a major challenge. In some regions, there is water scarcity, the residents use water from sources which may be unsafe. The separated system of water distribution had been broken hence both animal and human use the same water sources. The problem of declining health and sanitation (TNCIDP, 2017) may be due to lack of access to safe water. The study hence sought to assess health effects attributed to cyanotoxin poisoning from residents who use specific selected water sources.

2.3 Cyanotoxin levels

Cyanotoxins contamination in public water has become common (WHO, 2017). Reports of cyanotoxins poisoning incidents increases worldwide (EPA, 2019) hence becoming health hazards globally (WHO, 2019).

Different countries worldwide have their own threshold standards (WHO, 2017). New Zealand has set up guidelines for Cylindrospermopsin and for Microcystin at 1 µg/L (Health New Zealand, 2019). Canada guidelines for Microcystin is 1.5 µg/L (Health Canada, 2017). Australia values for cylindrospermopsin is 1 ug/L and 1.3 ug/L for microcystin-LR, while Finland has a value of 1 ug/L for microcystin. (Sanseverino *et al*, 2017).

The WHO recommended 0.7 µg/ L for Cylindrospermopsin and 1 µg/L for Microcystin-LR for drinking water (Chorus and Welker 2021). The values have been adopted by some countries, including Brazil, Czech Republic, France, Singapore and Uruguay. In Africa, South Africa has adopted the same value from WHO (Sanseverino *et al*, 2017).

The EPA views that cyanotoxins are found within the communities' unsafe water and not in specific lakes alone and the concern must be addressed (EPA, 2019). In areas where there is limited amount of rainfall, animals and human share the same water resource (WHO, 2022). The water may be unsafe for use due to water related diseases (WHO, 2019). Tharaka South Sub-County is a water scarce area and residents use water sources from reservoir (TNCIDP, 2017) which may be unsafe. This study established the level of cyanotoxins in man-made water sources to determine their safety in Tharaka Nithi County.

2.4 Physicochemical characteristics

The physicochemical characteristics of water is of important in cyanobacteria growth and hence cyanotoxins production (Dalu and Wasserman, 2018). The temperature, pH, Nitrogen, phosphorus and turbidity are indicators vital in cyanobacteria proliferation (Jahn *et al*, 2018).

Records have shown that high temperature, above 20°C (WHO, 2017) increases the cyanobacterial dominance (Schulhof *et al*, 2019). During summer, the percentage of cyanobacteria increases as the temperature increase (Gilbert *et al*, 2020). Temperature with nutrients promotes mineralization leading to proliferation of cyanobacteria (Schulhof *et al*, 2019). Extended warm periods provide conducive environment for cyanotoxins (Miller *et al*, 2017). Tharaka South Sub County experiences long warm periods without rain (TNCIDP, 2017) and hence water sources can be exposed to cyanotoxin production. The study determined temperature and compared it to the level of cyanotoxin in man-made water source to determine if it's a contributor

Cyanobacteria growth has been observed to increase with a pH range of 6-7 which makes blooms to occur and persist (WHO, 2017). pH promotes proliferation of cyanobacteria hence production of cyanotoxins (EPA, 2019). The study determined levels of pH with the level of cyanotoxin in man-made water sources in Tharaka Nithi County

Nutrients play major roles in cyanobacteria growth (ITRC, 2021). The nutrients include nitrogen and phosphorous (Wurtsbaugh *et al*, 2019) and are major contributors in cyanobacteria proliferations (ITRC, 2021). Sources of nutrients can be from fertilizers in the farm during storm runoff (Doubek and Carey, 2017) and from organisms or particles within the mat (Tee *et al*, 2020). Occurrence is most common in swallow waters (Huisman *et al*, 2018). Tharaka-Nithi County has swallow water reservoirs along which cultivations occurs and also from which animals draws water. This may lead to nutrients enrichment causing cyanobacteria proliferations. The study sought to

determine levels of total nitrogen and total phosphorus in the selected water sources to compare with the level of cyanotoxins

2.5 Sociodemographic factors influence selection of water source

Access to improved water sources reduces the cost of treating illnesses (World Bank, 2019) and better school attendance (WHO and UNCF, 2017). Accessing quality drinking water is a challenge to many poor rural settings (United Nations, 2018a) compared to urban areas (GSS, 2018). In Africa, access to improved water sources depends on place and wealth status (Armah *et al*, 2018). The majority of residents in rural areas can't access clean water (FAO, 2017) hence risking populations to water related disease (WHO, 2019). It's estimated that in every 10 persons, 4 lack access to improved drinking water in rural settings (WHO and UNICEF, 2017). This crisis happens where poverty and water inequity are endemic (United Nations, 2018b), for instance in Sub-Saharan Africa rural settings (World Bank, 2018).

Water scarcity also in the rural settings compels residents to depend on surface water (Umesh and Nagarkatte, 2017). In Ghana, 9% of rural settings have access to limited water, 6% unimproved water and 16% use surface water (GSS, 2018). Education, age, gender and distance also affects the access to improved water source (Abubakar, 2019)

Knowledge and information about water is important especially in rural areas to ensure good health, this can be made possible by understanding the quality of water sources and socio-demographic situations. This study closes that knowledge gap by interrogating the connection between socio-demographic factors and access to water source with particular focus on disadvantaged households from the Semi-Arid region.

It also established the effects of socio-demographic factors to determine water source in Tharaka South Subcounty

2.6 Methods for cyanotoxin detection

There are different methods on detection of cyanotoxins but HPLC is widely used. It's coupled with UV, PDA, LC-Mc among others. Being highly sensitive, HPLC is used in qualitative and quantitative analysis of cyanotoxins. Samples are extracted for concentration and purification. HPLC PDA was preferred in the study as it provides a measure of resolution of the congeners present (EPA, 2019).

2.7 Research gaps isolation

The study sought to fill the gaps which exists in Tharaka-Nithi county. The health effects attributed to cyanotoxin had not been assessed in the region. Investigation on the occurrence of cyanotoxins levels in surface water had not been established. The physicochemical characteristics that contribute to cyanobacteria growth had not been determined and the study also interrogated the connection between socio-demographic factors and the choice of the water source with particular focus on disadvantaged households from the Tharaka South Sub County which had not been established.

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CHAPTER THREE: METHODOLOGY

3.1 Study Area

The study was conducted in Tharaka South Sub-County in Tharaka Nithi County (Appendix I). The area comprises of 766.1 square kilometers and has a population of approximately 75,250. The average temperature ranges between 30°C to 35.9°C with an average rainfall of 500 to 800 mm/year which is experienced in month of March to May and October to December (GOK, 2019). The Sub-County was purposively selected because it experiences long periods without rain compared to the neighboring Sub-Counties and majority of residents depends on surface water for domestic use. The economic activities in Sub-County includes farming (millet, sorghum, cow peas), livestock rearing (goats, cattle) and mining (TNCIDP, 2017)

3.2 Study Design

Analytical cross-sectional study design was used because the relationship between cyanotoxin poisoning related health problems, cyanotoxins levels, physicochemical parameters and water source were examined as collectively confined in one place at a specific period (exposure and outcomes were both measured at the same time).

3.3 Measurement of variables

The dependent variable was the presence or absence of reported cyanotoxin poisoning health challenges. The category was between those with no symptoms, those with one symptom and those who had more than one symptom. It was measured on percentage levels and reported as 0.0%-24.9% (low), 25.0%-49.9% (high), 50.0% and above (very high).

The independent variables were the cyanotoxins levels, physicochemical characteristics and water source. Cyanotoxins (were measured by HPLC PDA in

micrograms and reported as high when exceeded 1 µg/L (WHO, 2017). At least two identical chromatogram peaks were selected in the analysis (EPA, 2015). In each physicochemical parameters, the average of the three analysis was used (EPA, 2015); pH (was measured by pH meter and indicated as low, 1-6, neutral, 7, and high, 8-14 (USGS, 2019). Temperature (was measured by thermometer and reported on scale of degree Celsius, low being $\leq 19^{\circ}$ and high being $\geq 20^{\circ}$ (WHO, 2017). Turbidity (was measured by turbid meters in NTU and reported as low, ≤ 10 NTU and high being ≥ 11 NTU (DataStream Initiative, 2021). Nutrients were measured using spectrophotometers in milligrams per liter and reported as high or low-level basing on WHO, (2017) scale. Socio-demographic factors were intermediate variables and measured as percentage. They were reported as high or low on comparison basis with other locations of study.

3.4 Study Population

The study targeted household heads or other dependable adults in the sampled households in the Sub- County as the respondents. Man-Made water sources included Earth dams, Water pans, Rock catchments and pipe water. Piped water and respondents in Marimanti who use piped water were used as a control point (Mugenda, Mugenda, 2012). Piped water subjected to treatment removes cyanotoxin through coagulation, clarification and filtration (EPA, 2016)

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion Criteria

Those who qualified for the study were the residents of Tharaka South Sub-County, household heads or any other dependable adult present (18 years and above for experience and knowledge) in the household at the time of the study, had been using

selected water for sampling and were willing to participate in the study.

3.5.2 Exclusion Criteria

The study excluded those who met the inclusion criteria but were absent at the time of the interview or were unwilling to participate in the study. Water sources were excluded on the following criteria; if they were dry or under renovation.

3.6 Sample Size Determination

The sample size for respondents was calculated using Fisher *et al*, (1998)

$$n = z^2pq/d^2 \quad \text{where;}$$

n = desired sample size

z = standard normal deviate (1.96 at 95% confidence level)

p = 0.5 (prevalence)

q = 1 - p = 1 - 0.5 = 0.5

d = degree of accuracy desired = 0.05 (statistically tolerated error)

Sample size =

$$n = \frac{(1.96^2)(0.50)(0.50)}{(0.05)^2} = 384 \text{ households}$$

For a population less than 10000, $nf = \frac{n}{1+n/N}$

N was estimated sample size, nf= sample size (n < 10,000)

$$nf = \frac{384}{1 + 384/5480} = 358$$

To cater for non-response, 5% of the sample was added making it to 375. Sample size of 375 households was obtained by proportionately sampling number of households using the selected water source. For example, those using Kaiboche water;

$$\frac{420}{5480} \times 375 = 29$$

The five water reservoirs for sampling were determined by calculating 30 % of 17 (30% x 17 = 5) (Mugenda and Mugenda, 1999). The tap water from Marimanti was included as a control point (Mugenda and Mugenda, 2012). In each water source, three samples were collected for each parameter to be analyzed, EPA recommends at least two samples for analysis (EPA, 2015).

Water Source	Approximate no. of households	Sample no. of households	Sampling Interval
Kaiboche	420	29	13
Gituma	500	35	11
Ntugi	560	38	10
Kijii	600	41	9
Kaguuma	1200	82	5
Marimanti	2200	150	3
Total	5480	375	-

Table 3. 1 Sampling frame

3.7 Sampling Technique

Systematic random sampling technique was used for households. A household to start with was randomly picked and the calculated interval followed in a chosen direction until the required number of households was reached

Sampling interval = N/n where;

N = Total number of sample size (375 households)

n = sample size for households using selected water source

Example for Kaiboche, sampling interval = $375/29$ = every 13th household

Simple random sampling was used to select 5 of 17 reservoirs water and 1 treated pipe water from 3 treated piped water (as control point).

For water sampling procedure (Appendix IV) in water reservoirs, two transect lines, A and B, intersecting at right angles were established to give sixteen sampling points positioned at equal distance from each (figure 2). Grab samples were obtained from these sampling points with a sampler to give sixteen samples, which were mixed gently to create a composite sample from which three samples of 500 ml were collected in containers for each parameter and labeled well (EPA, 2017a). For tap water, samples were obtained from different taps, mixed together to give a composite sample from which three samples of 500 ml were collected in containers for each parameter and labeled well. Samplers and containers were cleaned with distilled water and dried well throughout the processes (EPA, 2017a).

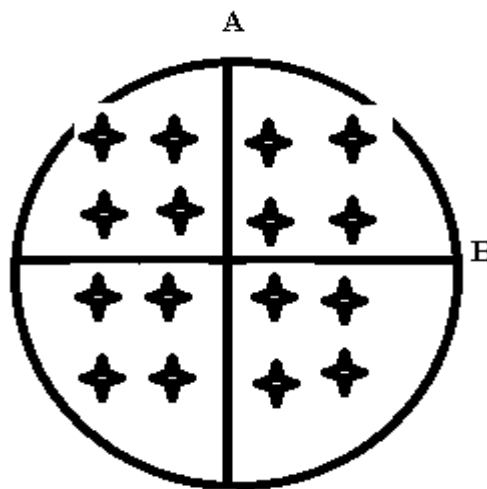


Figure 2 Water sampling points

Amber glass containers were used for cyanotoxin samples, they were placed in a cooler box at 6°C and kept in the dark within 20 minutes of collection to avoid photochemical and microbiological degradation (EPA, 2019). Plastic bottles containers were used for

Total nitrates and Total phosphorous samples. Temperature, pH and turbidity were tested in-situ while the other samples were transported to laboratory for analysis within 24 hours of sampling (EPA, 2017a)

3.8 Research Instruments

Interviews (Appendix III) provided information on cyanotoxin poisoning related health problems and the sociodemographic factors. This ensured more information was captured and confidentiality increased the willingness of the respondents to participate. Appendix V gives procedure for analysis of cyanotoxins and physicochemical. HPLC PDA analyzed cyanotoxins. Spectrophotometer determined Total nitrate and Total phosphorous. Turbidimeter determined turbidity. pH meter measured pH and temperature was tested by thermometer.

3.9 Pretesting of research instruments

Survey tools were pre-tested in Mukothima, Tharaka North Sub-County. The area was chosen as it has the same climatic characteristics as Tharaka south Sub-County. The water sampling tools and analysis tools were also subjected to pretesting.

3.10 Validity and Reliability

3.10.1 Validity

Validity refers to how well a tool measures what it is purposed to measure. Expert review of the instrument was done before commencement of the study. The researcher ensured that the questions were clear, properly grouped as per subject and also that instructions were easy to understand and follow. Research assistants were trained using standardized material to ensure they understood the operational definitions of the study and that they had a uniform approach to the respondents. The water samples were handled and protected guided by EPA procedures (EPA, 2017a).

3.10.2 Reliability

Refers to the degree to which an assessment tool produces stable and consistent results. This was ensured by pretesting of research instrument prior to data collection. This helped find out if sufficient response categories were available, whether participants were able to understand the questions and instructions and whether the questions meant the same for all respondents. The necessary adjustments were then made. For reliability analysis Cronbach's alpha was calculated by application of SPSS. All the alpha coefficients were greater than 0.7, therefore a conclusion was drawn that the instrument had an acceptable reliability coefficient and was appropriate for the study. Laboratory rules, technical advice from experts on carrying out the analysis and correct procedures from EPA was followed.

3.11 Data collection procedures

Respondents were contacted within the study area. Informed consent forms were acquired after which the researcher interviewed the respondent. Water sampling was done from 10:00 am to 12:00 pm. All the required procedural standards were followed in water sampling and analysis

3.12 Data analysis and presentation

Data was entered into SPSS version 21 for data management after being cleaned and coded. Frequencies and percentages were used to describe quantitative data. Pearson correlation was used to show association between disease symptoms related to cyanotoxins poisoning and cyanotoxin levels, and between cyanotoxins levels and physicochemical characteristics. Chi-square was used to show the association between the choice of water source and the sociodemographic factors. A P-value less than 0.05

showed a significant association between the variables. Results were presented in tables and bar charts

3.13 Ethical Consideration

Authorization to conduct the study was obtained from Kenyatta University, Graduate School. Ethical clearance to carry out the study was sought from KU-ERC (Kenyatta University Ethics and Review Committee) The research permit was sought from NACOSTI (National Council for Science Technology and Innovation), Nairobi, and Tharaka Nithi County office. All participants gave their Informed consent and confidentiality of the information collected was maintained. Data was disaggregated in a way that could not identify individuals. Respondents were also given clear information about the study to be able to choose to or not to participate. The respondents were also assured that there shall not be any form of victimization on their response and they were also made aware that they could withdraw at any time from the study.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results and discussion of the study in the following format; The socio-demographic characteristics of the respondents, the cyanotoxin poisoning related health problems, the cyanotoxin levels in water sources, physico-chemical characteristics in water sources and discussion

4.2 Socio-demographic characteristics of the respondents

Table 4.1 presents the socio-demographic characteristics of the respondents. The results indicate that majority of the respondents (54.40%) were of age 46 and above years and the minority (20.00%) were of age 18-36 years. Majority of the respondents (54.93%) had primary level of education and the minority (4.26%.) had not attended any level. Majority of respondents (48.53%) earn less than KShs 10000 per month and minority (22.67%) earns more than KShs 20000

Age	Frequency	Percentage
18-35	75	20.00
36-45	96	25.60
46>	204	54.40
Total	375	100

Education level		
Primary	206	54.93
Secondary	81	21.60
Tertiary	72	19.20
Never went to school	16	4.26
Total	375	100

Monthly income (approximate)		
Less than KShs 10,000	182	48.53
KShs 10,001-20,000	108	28.80
More than 20,001	85	22.67
Total	375	100

Table 4. 1 Socio-demographic characteristics of the respondents

4.3 Cyanotoxin poisoning related health problems

Table 4.2 presents cyanotoxin poisoning related health problems reported by the respondents. The results indicate that majority (36.59%) of the respondents with more than one disease symptoms related to cyanotoxins use water from Kijii followed by those who use water from Kaiboche (31.03%), Gituma (25.71%), Kaguuma (12.20%) and Ntugi (7.89%). Minority (7.33%) with more than one disease symptoms related to cyanotoxins poisoning were those using water from Marimanti pipe water.

Water Source	Those without symptoms	Those with one symptom	Those with more than one symptom		
Kijii					
Count	17 (41.45%)	9 (21.96%)	4SR 3ER 2GR	15 (36.59%)	6(ER, SR, GR) 5(JP, ER, SR) 3(SR, TR GR) 1(NV, TR)
Kaiboche					
Count	14 (48.28%)	6 (20.69%)	3SR 1ER 1JP 1GR	9 (31.03%)	5(TR, SR, NV) 2(ER, GR, SR) 1(ER, SR, TR,) 1(JP, NV)
Gituma					
Count	18 (51.43%)	8 (22.86%)	3SR 3ER 2GR	9 (25.71%)	5(ER, GR, SR) 3(ER, SR, NV) 1(JP, ER, TR)
Kaguuma					
Count	60 (73.17%)	12 (14.63%)	7GR 4SR 1JP	10 (12.20%)	6 (GR, SR) 4(ER, SR)
Ntugi					
Count	29 (76.32%)	6 (15.79%)	3GR 3ER	3 (7.89%)	3(GR, SR)
Marimanti					
Count	132 (88.00%)	7 (4.67%)	7GR	11 (7.33%)	5(GR, SR) 6(GR, JP)

ER (Eye Related), GR (Gastrointestinal Related), JP (Joint Pain), SR (Skin Related), TR (Throat Related), NV (Nausea and Vomiting)

Table 4. 2 Cyanotoxin poisoning related health problems reported by residents

4.4 The cyanotoxin levels in water sources

Table 4.3 presents results of cyanotoxins levels from the selected water sources. The results indicated that the highest level of cyanotoxin was in Kijii (3.6µg/L) followed by Kaiboche (2.3 µg/L) and Gituma (0.1µg/L). Kijii and Kaiboche had significant level above recommended 1µg/L by WHO (WHO, 2017). Cyanotoxins were not detected in Kaaguma, Ntugi and in Marimanti water sources.

Water Source	Type	Microcystin-LR (µg/L)	WHO standard (µg/L)
Kijii	Earthdam	3.6	1
Kaiboche	Rock-catchment	2.3	1
Gituma	Earthdam	0.1	1
Kaguuma	Water pan	nd	1
Ntugi	Water pan	nd	1
Marimanti	Tap water	nd	1

Table 4. 3 The cyanotoxin levels in water sources

4.5 The physicochemical characteristics in water sources

Table 4.4 presents results of physicochemical characteristics of the selected man-made water sources. The results indicated that the water samples had total nitrogen ranging from 2.14mg/l to 7.01mg/l (Kijii with highest while Ntugi with lowest); total phosphorous ranged from 0.98 mg/l to 2.16 mg/l, (Kijii with highest while Marimanti with lowest); pH value ranged from 7.8 to 9.8 (Kaiboche with highest while Marimanti with lowest); turbidity ranged from 6.4 to 21.2 NTU and temperature ranged from 18°C to 27.6°C (Kijii with highest while Marimanti with lowest in both).

Source of water	Cyanot (µg/L)	TN (Mg/L)	TP (Mg/L)	pH	Tur (NTU)	T °C
Kijii	3.6	7.01	2.16	9.3	21.2	27.6
Kaiboche	2.3	5.44	1.85	9.8	20.8	26.3
Gituma	0.1	6.11	1.88	8.6	20.0	26.9
Kaguuma	nd	3.2	1.02	8.3	11.0	21.7
Ntugi	nd	2.14	1.42	8.7	13.4	19.3
Marimanti	nd	2.47	0.98	7.8	6.4	18.0

Table 4. 4 Physicochemical characteristics in water sources

4.6 Comparison of cyanotoxins poisoning health effects and cyanotoxins levels

Figure 3 compares the percentage respondents with more than one disease symptoms related to cyanotoxins poisoning and cyanotoxin levels in selected water sources. The comparison shows that majority (36.59%) of the respondents with more than one disease symptoms related to cyanotoxins were those using water from Kijii which had high level of cyanotoxins (3.6 µg/L). Minority (7.33%) with more than one disease symptoms related to cyanotoxins poisoning were those using water from Marimanti pipe water where cyanotoxins were not detected

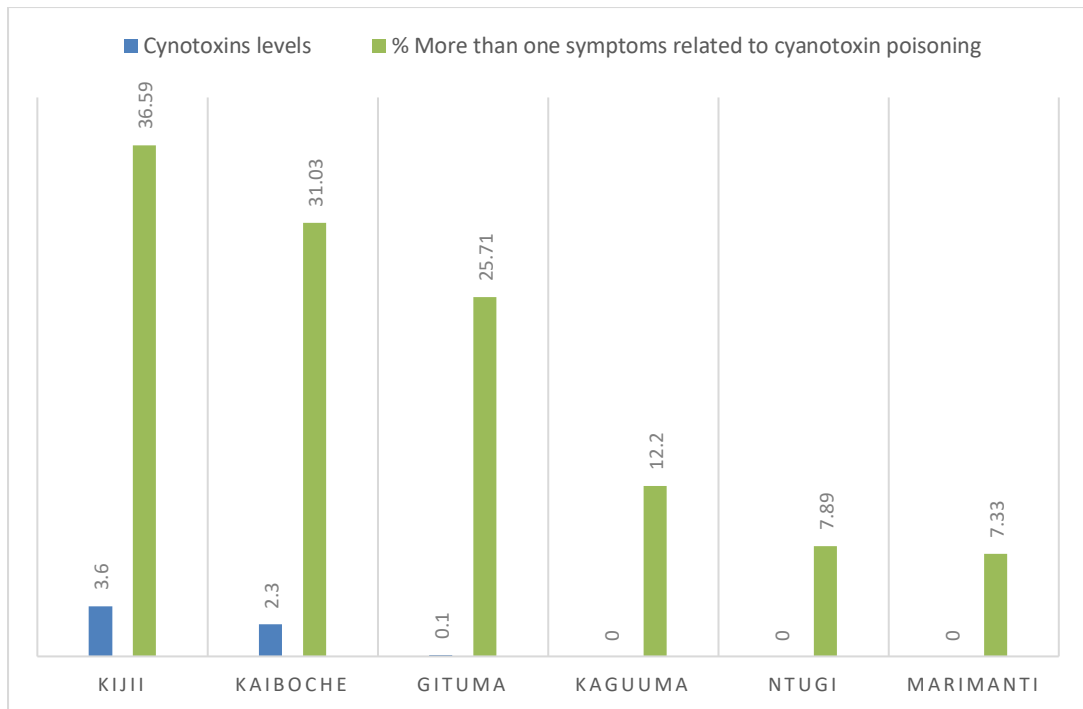


Figure 3 Symptoms related to cyanotoxins poisoning and cyanotoxins levels

4.7 Chi-square of socio-demographic factors and choice of the water source

Table 4.5 presents the association between the socio-demographic factors and the choice of the water source. A p-value <0.05 showed that there was a significant association while $p>0.05$ showed that there was no significant association. The results showed that age and level of education had no significant association (p-value >0.05) while income levels had an influence (p-value <0.05)

Independent variable	% Not support the use of selected water	% Support the use of selected water	Chi-square	P-value@95% CL	Df
Age					
18-35	4(8.3%)	6(10.5%)	63.74	0.199	4
36-45	16(33.3%)	26(45.6%)			
46-	28(58.3%)	25(43.9%)			
Level of education					
Primary & never went to school	36(75.0%)	35(61.4%)	60.32	0.213	9
Secondary	12(75.0%)	19(33.3%)			
Tertiary	0	3(5.3%)			
Level of income					
<10,000	24(50.0%)	25(43.9%)	39	0.002	6
10,001-20,000	17(35.5%)	19(33.3%)			
>20,001	7(14.5%)	13(22.8%)			

Table 4. 5 Chi-square of socio-demographic factors and choice of the water source

4.8 Correlation of cyanotoxin poisoning related health effects and cyanotoxin levels

Table 4.6 presents the association between the disease symptoms related to cyanotoxin poisoning and cyanotoxins levels. A p-value <0.05 showed that there was a significant association while p>0.05 showed that there was no significant association. The results showed that the correlation coefficient was strong (.859) with a p-value <0.05 and hence association between cyanotoxin poisoning related health effects and the cyanotoxin levels

Correlations

		cyanotoxin	%More than
cyanotoxins	Pearson	1	.859*
	Sig. (2-tailed)		.028
	N	6	6
%More than one symptom	Pearson	.859*	1
	Sig. (2-tailed)	.028	
	N	6	7

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4. 6 Correlation between cyanotoxin poisoning related health effects and cyanotoxin levels

4.9 Correlation between cyanotoxin levels and physicochemical characteristics

Table 4.7 presents the correlation between the cyanotoxin levels and physicochemical characteristics. A p-value <0.05 showed that there was a significant association while p>0.05 showed that there was no significant association. The results showed a strong correlation in all physicochemical indicators and the cyanotoxins; Total nitrogen (r = .746), Total phosphate (r = .760), pH (r = .786), Turbidity (r = .694) and Temperature (r = .696). A p-value <0.05 indicated a significant correlation between cyanotoxins and all physicochemical characteristics.

		Correlations					
		Cyanotoxin	Total Nitrogen	Total Phosphate	pH	Turbidity	Temperature
Cyanotoxin	Pearson Correlation	1	.746**	.760**	.786**	.694**	.696**
	Sig. (2-tailed)		<.001	<.001	<.001	.001	.001
Total Nitrogen	Pearson Correlation	.746**	1	.897**	.651**	.885**	.973**
	Sig. (2-tailed)	<.001		<.001	.003	<.001	<.001
Total Phosphate	Pearson Correlation	.760**	.897**	1	.798**	.960**	.892**
	Sig. (2-tailed)	<.001	<.001		<.001	<.001	<.001
pH	Pearson Correlation	.786**	.651**	.798**	1	.861**	.736**
	Sig. (2-tailed)	<.001	.003	<.001		<.001	<.001
Turbidity	Pearson Correlation	.694**	.885**	.960**	.861**	1	.940**
	Sig. (2-tailed)	.001	<.001	<.001	<.001		<.001
Temperature	Pearson Correlation	.696**	.973**	.892**	.736**	.940**	1
	Sig. (2-tailed)	.001	<.001	<.001	<.001	<.001	
N		18	18	18	18	18	18

** . Correlation is significant at the 0.01 level (2-tailed).

Table 4. 7 Correlation of cyanotoxin levels and physicochemical characteristics

4.10 Discussion

4.10.1 Socio-demographic factors

It was found out that income level influenced the choice of the water source. This was the anticipated finding, though the finding about lack of influence by the age and the educational levels on the choice of the water source was unforeseen. Chi-square test of association (Table 4.5) showed significant associations between income and the choice of the water source. Age and education level had no significant association to the choice of the water source. On income level, respondents with less than KShs 10000 per month contributed the largest percentage in support of the use of selected water source. This is most likely because poverty is related to level of income and agrees with (Wahaj *et al*, 2018) who found that poor people living in water scarce areas are unable to access safe water for their domestic purposes. In Nigeria, majority of

these people are from rural settings and end up using water from unimproved sources exposing them to water related diseases (Ishaku *et al*, 2011). Also in Nigeria, the trend to access safe water is attributed to financial aspects and the challenges are to those in rural settings who cannot access clean safe water supply (Egbinola, 2017). Socio-economic status is the main indicators to poor water and sanitation (Angoua *et al*, 2018). Distance to water point affects the access to safe water especially to those with low income and with a distance of more than a kilometer to water point (WHO and UNICEF, 2017). The respondents couldn't fetch clean water which is found in a long distance and they could not afford to buy clean water which is sold at KShs 30 per 20 liters. The majority of respondents who use water from the selected sources were older compared to those below 36 years. According to the respondents, they had no enough strength to fetch water from far because of their age. The majority of respondents had primary education compared to tertiary education. They claimed the lack of finance and poverty level made them not complete their education. It was then inevitable to guess that residents took the water from the sources because of their socioeconomic status, age and education without knowing the risks of cyanotoxins contaminations.

4.10.2 Cyanotoxin poisoning related health problems

This study aimed at establishing disease symptoms related to cyanotoxins poisoning in Tharaka South Sub-County. It was found out that the cyanotoxin levels in the water source influenced the cyanotoxin poisoning related health problems. The Pearson correlation (Table 4.6) showed significant associations between these factors. The study findings indicate that majority (36.59%) of the respondents with more than one disease symptoms related to cyanotoxins poisoning use water from Kijii, most likely because of the presence of the high level of cyanotoxins (3.6 µg/L) compared to other

water sources. Six respondents had eye irritation/reddening, skin irritation/rashes and gastrointestinal related symptoms. Five respondents had joint pain, eye irritation, skin irritation and abdominal pains. Three respondents had skin rashes, throat related and abdominal pains while one person had nausea/vomiting and throat related symptoms. This is quite comparable to Ohio, New York, where out of 61 victims, 8 had skin related symptoms, 8 gastrointestinal related symptoms and 3 eye related symptoms in 2009/2010 (Hilborn *et al*, 2014). This also compares with studies in Finland in 2010 where similar symptoms were recorded from recreation water contaminated with cyanotoxins where victims had gastrointestinal (53%), ear/eye/nose/throat irritation (29%), nausea (34%), and skin irritation (34%) symptoms (Berg *et al*, 2011). In 2011, similar symptoms were reported in Milford Lake (Kansas) where irritation of eyes and upper respiratory tract was quite high (71%) while nearly comparable to skin rashes (29%) and gastrointestinal (14%) related symptoms (Trevino *et al*, 2015). In 2014, 3.19 µg/L microcystin were detected in Ohio (Lucas County) with 16.2% of more than 100,000 households demonstrating nausea and vomiting, irritation of skin and eye, headaches, and respiratory symptoms (McCarty *et al*, 2016).

In Brazil, the finding was quite high where out of 130 patients, 116 demonstrated eye related and headache symptoms after exposure to microcystins contaminated surface water during dialysis (Hilborn *et al*, 2013). This agrees to the study in Canada (2009) and Serbia (2012-2015) where there were incidences of skin and gastrointestinal symptoms with cyanotoxin contaminated water (Lévesque *et al*, 2016, Svirčev *et al*, 2017) but it is high compared to the findings of a study in Argentina where only one person had symptoms related to cyanotoxins after exposure to contaminated recreation water 2007(Giannuzzi *et al*, 2011).

It was inevitable to guess that the symptoms recorded were caused by cyanotoxin poisoning. EPA associate abdominal pain, joint pain, nausea/vomiting, skin rashes/irritation and eye discharge/reddening/irritation to cyanotoxins (EPA, 2021). The symptoms were high in water sources with high cyanotoxins compared to sources where cyanotoxins were not detected. The residents directly contact water during fetching and when they use it in cooking, bathing and washing which may be the routes for cyanotoxin entry into the body (WHO, 2019). Respondents noted that symptoms occurred few days or weeks after using the water. The symptoms also persisted even after medical treatment. The studies support that health effects to cyanotoxin poisoning appears within minutes to days after contact with contaminated water (EPA 2019). This calls for quick intervention from the government to safe residences in Tharaka South.

4.10.3 Cyanotoxin levels in selected water source

The highest level of cyanotoxin were in Kijii water source (3.6g $\mu\text{g/L}$) followed by that in Kaiboche (2.3 $\mu\text{g/L}$) and Gituma (0.1 $\mu\text{g/L}$). The two sources, Kijii and Kaiboche had significant level above WHO (1 $\mu\text{g/L}$) recommendation (WHO, 2017). Cyanotoxins were not detected in Kaaguma, Ntugi and Marimanti water sources. The microcystin-LR detected in Kijii and Kaiboche water sources were much higher compared to the ones detected in Great Lakes (0.084 $\mu\text{g/L}$ and 0.076 $\mu\text{g/L}$) in 2004 (Makarewicz *et al*, 2006). This was also higher than that found in Ethiopia seven lakes where microcystin-LR were detected in three lakes (1.0 $\mu\text{g/L}$ in Chamo, 0.7 $\mu\text{g/L}$ in Langano and 0.6 $\mu\text{g/L}$ in Ziway) (Eva *et al*, 2011). This was also quite high compared with Mhlanga *et al*, (2006) who recorded 0.1–1.6 $\mu\text{g/L}$ in Chivero Zimbabwe in 2003–2004. In Utinga Reservoir in 1999, 0–1.25 $\mu\text{g/L}$ microcystin were detected (Vieira *et al*, 2005). In Kenya, microcystin-LR were detected in lake Baringo in June, 2001 (2.98

µg/L), January (0.75 µg/L) and May (0.08 µg/L) in 2002 (Ballot *et al*, 2003). Mwaura *et al*, (2004) detected microcystin in a range of 0.06 - 2.85 µg/L. Microcystin were also detected (0–1.7 µg/L) in Oxidation ponds in 2001–2006 (Kotut *et al*, 2010). Ballot *et al*, (2003) also recorded microcystin (3.25 µg/L) in November 2001 which was quite comparable to this study. High cyanotoxins have also been detected in various parts of the world compared to this study. Nguyen *et al* (2007) recorded a range of 0-76 µg/L in Various water bodies in Vietnam. In Brazil, 28–45 µg/L were detected in Monjolinho reservoir in 2004 (Sotero-Santos *et al*, 2008). In lake Koka, 28 µg/L were detected in Ethiopia (Eva *et al*, 2011)

It was found out that the Physicochemical characteristics influenced the cyanotoxin levels in water. The Pearson correlation (Table 4.7) showed significant associations between these factors and the cyanotoxin levels. It was noted that the sources with high levels of cyanotoxin had also high physico-chemical parameters compared to the one with low or no cyanotoxins. Kijii water source which had highest (3.6 µg/L) level of cyanotoxins corresponded with high levels of physicochemical characteristics; Total nitrogen (7.01mg/l), Total phosphorous (2.16mg/l), pH (9.3) turbidity (21.2 NTU), and Temperature (27.6) compared to Marimanti water pipe water which had no cyanotoxins and had the lowest physicochemical parameters; Total nitrogen (2.47mg/l), Total phosphorous (0.98mg/l), pH (7.8), turbidity (6.4 NTU), and Temperature (18°C). This was in line with studies in Lake Naivasha (Boar *et al*, 1999) and Lake Victoria (Verschuren *et al*, 2002) which had high nutrients. It was inevitable to guess that water reservoirs are exposed to nutrients from land degradation, wildlife, cultivation, domestic animals and rising demographic pressure. These factors have potential to promote cyanotoxins (WHO, 2022). WHO (2017), Carey *et al*, (2012), Kosten *et al*,

(2011) and Wang *et al*, (2002) demonstrated that cyanotoxins proliferation occurs in relation to physicochemical characteristics. Presence of cyanotoxins is a cause of concern and a potential risk to cyanotoxin poisoning. The cyanotoxins concentrations above the acceptable limit (1.0ug/L) in water (WHO, 2017) poses a challenge to the residents of Tharaka South Sub-County.

4.10.4 Physicochemical characteristics in selected water source

Total nitrogen ranged from 2.14mg/l to 7.01mg/l. Total phosphorous ranged from 0.98 mg/l to 2.16 mg/l. pH value ranged from 7.8 to 9.8. Turbidity ranged from 6.4 to 21.2 NTU and temperature ranged from 18°C to 27.6°C. This was similar to physicochemical parameters found in Zaria, Nigeria where it was recorded temperature of 27.63 ± 1.09 , pH of 6.30 ± 0.11 , phosphorous phosphates (mg L⁻¹) 242.00 ± 74.94 , Nitrate nitrogen (mg L⁻¹) 53.25 ± 18.03 . There was also positive relationship with the cyanobacteria growth (Mathias *et al*, 2013). Fosso-Kankeu *et al*, (2008) recorded temperature from 27°C to 29°C and a pH of 6.33 which had influence on microcystin production. In Nakuru maturation pond, similar physicochemical were associated with the presence of microcystins in the pond where temperature ranged between 20.5-28.5 °C, pH 10.4- 8.8, Total nitrogen(mg/L) 4.0- 8.0 and Total phosphorus(mg/L) 1.4- 5.2 (Kotut *et al*, 2010). In Lake Baringo, physicochemical parameters tested in different months in 2001 and 2002 had quite comparable physicochemical characters; Temperature (°C) ranged from 23.7 - 26.3, pH ranged from 8.8 -9.1, Total Nitrogen (mg/ L) ranged from 0.5-8.0 and Total Phosphorous (mg/L) ranged from 0.6 - 1.3 (Ballot *et al*, 2003).

Environmental factor plays major roles in the cyanobacteria growth and cyanotoxin

production (Lilover and Stips, 2008). Total nitrogen, total phosphates, pH, turbidity and temperature, influences the growth of cyanobacteria (Paerl & Otten, 2013). Majority of water sources that had high physicochemical indicators had also high levels of cyanotoxins. According to Ma *et al*, (2014), total nitrogen of 3.1 to 49.6 mg/L promotes the cyanobacteria and cyanotoxins production. Apart from Marimanti and Ntugi, all the sources of water had a threshold above 3.1 mg/L an indicative of cyanobacteria growth. A threshold of 25 µg/L of total phosphorous concentrations is capable of causing cyanotoxins (WHO, 2003). All the water sources had higher level of total phosphorous compared to this threshold but other physicochemical characteristics could have contributed to cyanotoxins production in sources detected. Nitrates or phosphates in water causes eutrophication and anoxic environment leading to cyanotoxin production (Paerl & Otten, 2013). It was inevitable to guess that the nutrients are caused by human activities and natural forces around the reservoirs. Animal wastes and runoff could also be loading nitrogen and phosphorus in water which may be promoting cyanotoxin production. According to CRS, (2019), these factors promote cyanobacteria growth.

pH value ranged from 7.8 to 9.8, Kaiboche having the highest level while Ntugi lowest. According to WHO, a pH range of 6 to 9 promotes the cyanobacteria growth (WHO, 2003). A pH of 9.1 to 10.4 increased microcystin in Nakuru town waste water treatment facility (Ja'hnichen *et al*, 2001). Zohary & Robarts, (1987) documented high pH values in Barwon-Darling River in Australia. The correlation between pH and cyanobacteria was shown in the Europe lakes (Kosten *et al*, 2011). The high pH in water is attributed with higher proportion of cyanobacteria photosynthetic rates caused by high nutrient concentrations that stimulate phytoplankton growth and bloom formation (Duy *et al*,

2000). Cyanobacteria's capacity to utilize CO₂ efficiently requires higher pH (Caraco and Miller, 1998). The recorded values in all water sources were within the range of cyanotoxin production, but detection was only recorded in Kijji, Kaiboche and Gituma showing that other environmental factors could have contributed to cyanotoxin production.

Turbidity ranged from 6.4 NTU to 21.2 NTU. The highest was seen in Kijji, Kaiboche and Gituma at 21.2, 20.8 and 20 NTU respectively. Turbidity greater than 10 NTU shows the presence of blooms which gives the water source turbid condition (WHO, 2003). When turbidity is low, more light can penetrate through the water creating favorable conditions for algal growth which in return, develops a turbid environment (WHO, 2003)

Temperature ranged from 18^oC to 27.6^oC. According to WHO 2003, the temperature between 20^oC and 30^oC promotes cyanobacteria growth. Kijji had higher temperature, 27.6^oC, followed by, Gituma 26.9^oC, and Kaiboche 26.3^oC. Marimanti had lowest temperature of 18^oC. The cyanotoxin in Kijji, Kaiboche and Gituma could have been promoted by high temperatures in their waters. The study agrees with EPA, (2015), WHO, (2017) and WHO, (2003) which recorded high proliferation of cyanobacteria in temperatures greater than 20^oC. Bowling & Baker, (1996) recorded a temperature of 25-30^oC which promoted growth of cyanotoxin producing bacteria in Barwon-Darling River in Australia. *Microcystis aeruginosa*, was also documented to grow faster at higher temperature in Japan by Kuwabara *et al*, (2001). The higher temperatures in Tharaka South could contribute to cyanotoxin production. Ja'hnichien *et al*, (2007)

points out that external factors like temperature between 24⁰C and 28.5 ⁰C increases microcystin production in *M. aeruginosa*.

CHAPTER FIVE: SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter presents the summary of major findings, conclusions and recommendations arranged as per the study objectives.

5.2 Summary

The study findings indicate that majority of the respondents (54.40%) were of age 46 and above years and the minority (20.00%) were of age 18-36 years. Majority of the respondents (54.93%) had primary level of education and the minority (4.26%.) had not attended any level. Majority of respondents (48.53%) earn less than KShs 10000 per month and minority (22.67%) earns more than 20000. Majority (36.59%) of the respondents with more than one disease symptoms related to cyanotoxins use water from Kijii which had high level of cyanotoxins (3.6µg/L) and minority (7.33%) were with those who use Marimanti tap water where cyanotoxins were not detected. Kijii and Kaiboche had a significant level of cyanotoxin above WHO recommended value (1µg/L). The physicochemical characteristics were high in sources with high cyanotoxins (Kijii) while low where cyanotoxins were not detected (Marimanti). Total nitrogen ranged from 2.14mg/l to 7.01mg/l; total phosphorous ranged from 0.98 mg/l to 2.16 mg/l; pH value ranged from 7.8 to 9.8; turbidity ranged from 6.4 to 21.2 NTU and temperature ranged from 18°C to 27.6°C. It was found out that income level of residents influenced the choice of water source while age and the educational levels did not have the influence. Cyanotoxin levels in the water source influenced the

disease symptoms related to cyanotoxins poisoning. Physicochemical characteristics influenced the cyanotoxin levels in water source in Tharaka South Sub-County.

5.3 Conclusion

5.3.1 Sociodemographic factors

- Income level of residents influenced the choice of water source. Age and the educational levels did not have the influence.
- The source of income for most residents is through farming, rearing of goats and cattle. This may take them more than three months to get cash and hence accessing clean water either through buying or fetching from a distance becomes difficult. This may force residents to continue using the water reservoirs which may risk them with cyanotoxin poisoning.
- Improving socioeconomic of residents by providing employment or source of sustainable income generating projects is essential. Providing improved infrastructures to store and supply clean water especially during rainy season will also help.

5.3.2 Cyanotoxin poisoning related health problems

- Cyanotoxin levels in the water source influenced the disease symptoms related to cyanotoxins poisoning.
- Residents contact water through fetching or drawing water, walking inside and swimming. This can expose them to cyanotoxin poisoning.
- Making health workers and residents aware of disease symptoms caused by cyanotoxins poisoning will improve the health of the residents by giving

correct medical advice and seeking alternative way of water if their water is contaminated by cyanotoxins

5.3.3 Cyanotoxin levels of water in Tharaka Nithi

- Physicochemical characteristics influenced the cyanotoxin levels
- Presence of cyanotoxins is a cause of concern and a potential problem especially level above WHO recommendation
- The residents appeared to be unaware of cyanotoxins in their water source. Therefore, making residents aware of cyanotoxin in water source is important.
- The safest and most advocated water quality is the one which has been treated.

5.3.4 Physicochemical characteristics of water in Tharaka Nithi

- Environmental factor plays major roles in the cyanobacteria growth which also affects cyanotoxin production
- The total nitrogen, total phosphates, pH, turbidity and temperature, influences the growth of cyanobacteria.
- Consuming nitrates can cause methemoglobinemia. Digestive problem could occur from extremely high level of phosphate. pH could lead to headaches while turbidity can lead gastrointestinal

5.4 Recommendations

5.4.1 Recommendations from the study.

- Government to address the challenge of water scarcity especially in rural settings, where residents are poor and unable to access clean water.
- The residents and medical practitioners be informed of cyanotoxin levels in water sources detected and attributed cyanotoxin poisoning.
- Cyanotoxins to be included in water quality standard by Kenya Bureau of

Standards

- Reservoirs water to be restricted areas to avoid excess nutrients from animals and farms.

5.4.2 Recommendations for further research

- A study to determine influence of gender, marital status, employment, religion and under 18 years on choice of water source in rural water scarce areas.
- A case-control study to establish if there is presence of cyanotoxins in blood systems of the residents.
- Studies to determine other types of cyanotoxins in water reservoirs like anatoxin-a and cylindrospermopsin.
- Experimental research on physicochemical versus cyanobacteria growth.
- Related study to be done in other parts of the country.

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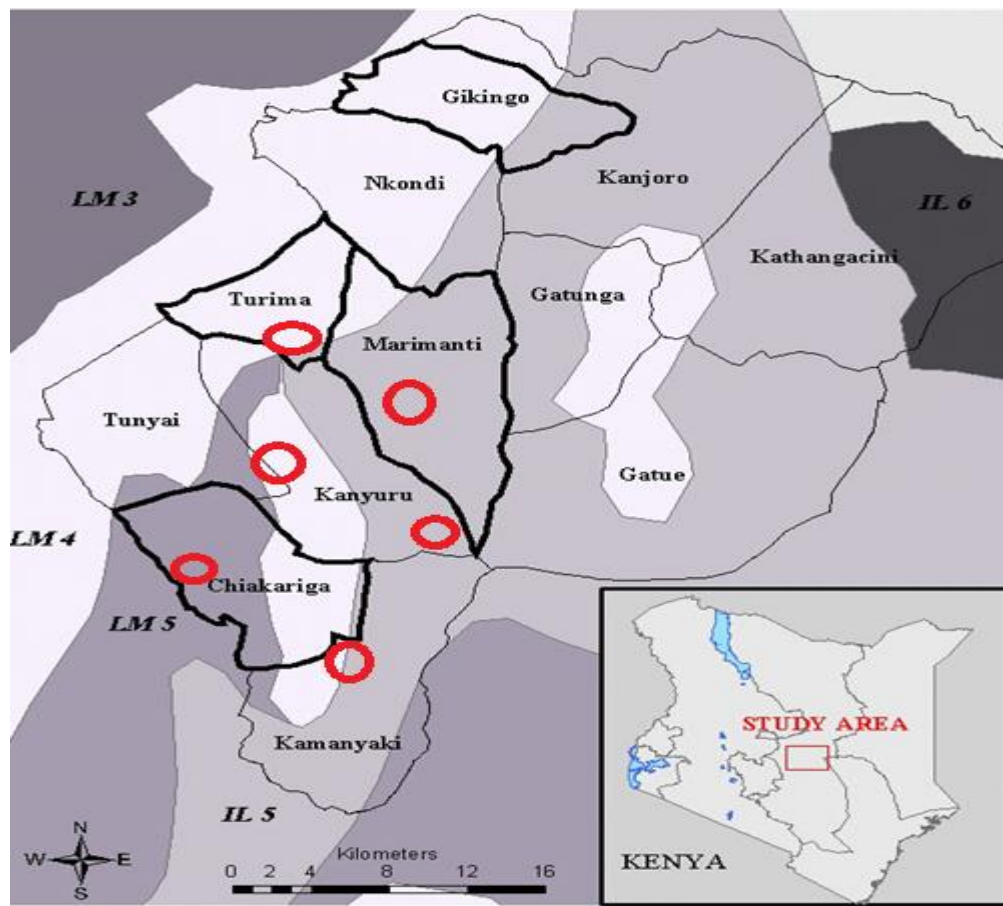
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LIST OF APPENDICES

APPENDIX I Map of Tharaka South Sub-County



APPENDIX II Informed consent form

I am Dickson Wakhungu pursuing Master of Science, Environmental Health, Kenyatta University. My study is on “Cyanotoxin levels in Man-made water sources and Health Related Conditions in Tharaka-Nithi County, Kenya”. The data from this study will help the ministry of public health in promoting health in this place & other parts of the Country.

Procedure to be followed

As participant, I kindly request that I ask you questions. I will have to record your response. You are also informed that care will be given equally for the participants and nonparticipants, so you have the choice to agree or refuse. In case you have a question, you will have freedom to ask at any particular time of the study.

You also have freedom to respond or refuse to give information as you wish, and you can still stop your participation during the interview time without any denial of any services given from the county or organization any time.

Discomforts and risks

During the interview, you may come across some responses which are discomfort like if you had some infections and which may seem to be embarrassing, kindly you are free not to respond or give information related to this. The time of interview will be taken at estimation of 30 minutes.

Benefits

You are important person in the study because you will help us learn, get information, and be able to understand more about man-made water are contributing to water related infection that can improved to reduce the water related infections.

Rewards

Participation in the interview/the study some refreshment may be provided at if you will be comfortable with them

Confidentiality

Your place of choice will be given priority. If you will be comfortable at your home place or any place you choose. Also note that no details of your name will be written on questionnaires. This documents also will not be exposed and will be safely locked

Contact information

If you have any questions you may contact Dr. Daniel Akunga, supervisor 1 on 0722552157 or Dr. Peterson Warutere, supervisor 2 on 0721993833 or the Kenyatta university ethical review committee secretariat on chairman.kuerc@ku.ac.ke , secretary.kuerc@ku.ac.keercku2008@gmail.com

Participant statement

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private that I can leave the study at any time. I understand that I will still get the same care whether I decide to leave the study or not and my decision will not change the care I will receive from the County.

Code of Participant.....Signature Date.....

Investigator statement

I, the undersigned, have explained to the volunteer in a language s/he understands the

procedures to be followed in the study and the risks and benefits involved.

NameSignature Date.....

APPENDIX III Data collection tools

Part 1: Interview

My name is DICKSON WEKESA WAKHUNGU pursuing a Master's degree in Kenyatta University. I am carrying out a study on "**CYANOTOXIN EXPOSURE AND ATTRIBUTABLE HEALTH EFFECTS AMONG RESIDENTS OF THARAKA-NITHI COUNTY, KENYA**" I request for 25 minutes of your time so that you can answer some questions in relation to the study. The information that you will give will be handled with utmost confidentiality and will be used solely for meeting the study objectives. You are free to withdraw from the study at any time, skip any questions or fail to answer any questions with no penalty. However, your opinion to this study is very important as it will help in policy enrichment by providing baseline information on water quality in Tharaka South Sub- County.

Respondent identification codeAddress.....

Mobile No.....

Section A: Socio-demographic information.

1. What is your age in years
2. Gender.....
3. For how long have you been a resident in in Tharaka South Sub-County..... weeks/ months/ years
4. What is your highest level of education attained.....
5. Are you employed..... If not employed, what's the source of your income.....
6. What is your approximate monthly income in the category of;

- a. Less than Kshs. 10,000
- b. 10,000- KShs 20,000
- c. More than KShs. 20,000

SECTION B. Activities with the water

- 1. Where does the water you use come from?
- 2. Do you treat this water before using? If yes, how do you treat the water?.....
- 3. Do you shower with the water from this site?.....
- 4. Do you drink water from this site?.....
- 5. Do you cook with this water?.....
- 6. Do you swim or play in the water?.....
- 7. Mention other activities you use with the water
- 8. Is the water source you use of good quality for your health?.....
- 9. Give the reason why you chose to use this water?

SECTION C. Information on symptoms related to cyanotoxins poisoning.

- 10. Have you have had any of the following ill health symptom after using the water
 - a. Skin rashes/Irritations.....
 - b. Sneezing/runny nose/ sore throat.....
 - c. Eye discharge/irritation/reddening.....
 - d. Abdominal pain or gastrointestinal pains.....
 - e. Nausea/ Vomiting.....
 - f. Muscle pains.....
 - g. Do you experience any other conditions related to the above symptoms?.....
.....
.....
- 11. How many, in number, stay in the house with you?.....
- 12. Do they all have similar problems, if any in the above Questions?.....
- 13. Do these symptoms reappear repeatedly or once?.....

- 14. Do these symptoms appear after using this water?
- 15. Have you heard of or seen blue green algae?.....If yes, how did you get to know them or where did you see them?.....
- 16. Do you know cyanotoxins?..... If yes, how did you get to know them?.....
- 17. Do you know any harm cyanotoxins can cause?

There is no question left. I want to thank you for the information. Your contribution is greatly appreciated!

APPENDIX IV Sampling Procedures for water

Sampling from Reservoirs:

- Exact sampling point (including depth) were determined for water reservoirs
- Samplers were used to be collect water samples at a specified depth
- Sample containers were filled to ¼ inch from the top and capped
- Exterior of the container were wiped off
- Labelling was done by recording the sample identification number, date and time of sample collection, sample location, weather, and information on water conditions with a permanent marker. Labels were covered with clear tape so that the fresh ink should not wash off or smear.
- Sample documentation form(s) were filled appropriate
- All PPE and wastes were handled careful placed into a garbage bag for proper disposal procedures

Sampling from tap water;

- Aerator / screen or any dirt from the tap were removed.
- Water was run for 5 minutes
- flow from the tap was adjusted to about a ¼ inch diameter
- Flow was maintained in a steady flow of water until the water temperature was constant
- Sample container was hold under the discharge at an angle so that the sample flows down the inside wall of the sample container to minimize aeration.
- sampling containers were filled directly from the tap to the marked line

APPENDIX V Laboratory analysis procedures

Analysis of cyanotoxin by HPLC-PDA

Reagents; gas (Helium), Eluent (A) & (B), water + TFA, TFA (0.5 ml) mixed with 1,000 ml(water) acetonitrile + TFA, Methanol, HPLC grade, standard

Procedure:

1. Degas (in helium gas stream) & Solvents were Prepared
2. Time in minutes (0 10 40 42 44 46 55), Eluent A (70 65 30 0 0 70 70) % B (30 35 70 100 100 30 30) % linear gradient 1 ml/min was Programed
- 3 Desired temperature of the column and starting gradient conditions was equilibrated
4. PDA was set at 200- 300 nm for monitoring.
5. Firstly running of blank sample was done (injecting methanol)
6. Filtering of samples was done for particulates removal.
7. Retention time was indicated by running a standard at the beginning, this was also repeated at the end.
8. performing of calibration curve was done regularly at intervals, (when lamp was changed, replacing the column).
9. Observation of chromatograms & integration was done at 238 nm

Procedure for photoelectric turbidimeter operation

The turbidimeter was connected to mains, switched on to warm up for about 5 minutes.

The water sample was poured whose turbidity was to be determined into the glass cell provided (two identical; one to hold the sample and the other standard. The standard which has the closest turbidity to that of the sample was compared visually

Total Nitrogen determination

Equipments and reagents

Glass culture tubes, 10 mL micropipette, Repipettes, Digestion Solution, H₂SO₄, Analytical Balance, Autoclave, Centrifuge, Spectrophotometer, Sodium Hydroxide, Potassium Peroxydisulfate, Sulfuric Acid, Nitrate Standard Solution, Ammonium Standard Solution

- 10 mL aliquots of well mixed water were put into acid washed culture tubes.
- Sodium hydroxide and potassium persulfate were added, autoclaved to oxidize and convert all of the nitrogen derivatives into nitrate.
- After the digestion process was completed, tubes were acidified and centrifuged prior to analysis.
- Smaller sample was placed in a vial and color-forming reagents was added (this forms light/dark pink color)
- A spectrophotometer was used to run an absorption analysis to determine the TN level at three wavelengths (230, 225 and 220 nm).
- The second derivative of the three absorbency values was related to the nitrate concentration in the sample.

Total phosphorous determination

Equipments and reagents

10mL of each phosphorus calibration sample (0:0625, 0:125, 0:25, 0:5, and 1mg=L)

10mL of sample, 90mL reagent, cuvettes, 10mL graduated cylinder, DI Water

Orthophosphates was reacted with ammonium heptamolybdate to form a phosphomolybdic acid. The complex was then reduced by ascorbic acid in the presence of potassium antimony tartrate to form molybdenum blue which was measured

- Graduated cylinders and cuvettes were rinsed with DI water.
- 6ml of reagents and 6ml of calibration sample were added to cuvette
- 6 mL of DI water and 6 mL of reagent were added to the blank
- Solutions were allowed 30 minutes for the color to develop.
- Spectrophotometer was run
- Absorptivity for the blank and calibration samples was recorded
- Record absorptivities for the water samples in another table
- Calibration curve was done (absorptivity versus concentration)
- Determination of the equation of best linear was done

Procedure for potentiometric pH test

Reagents

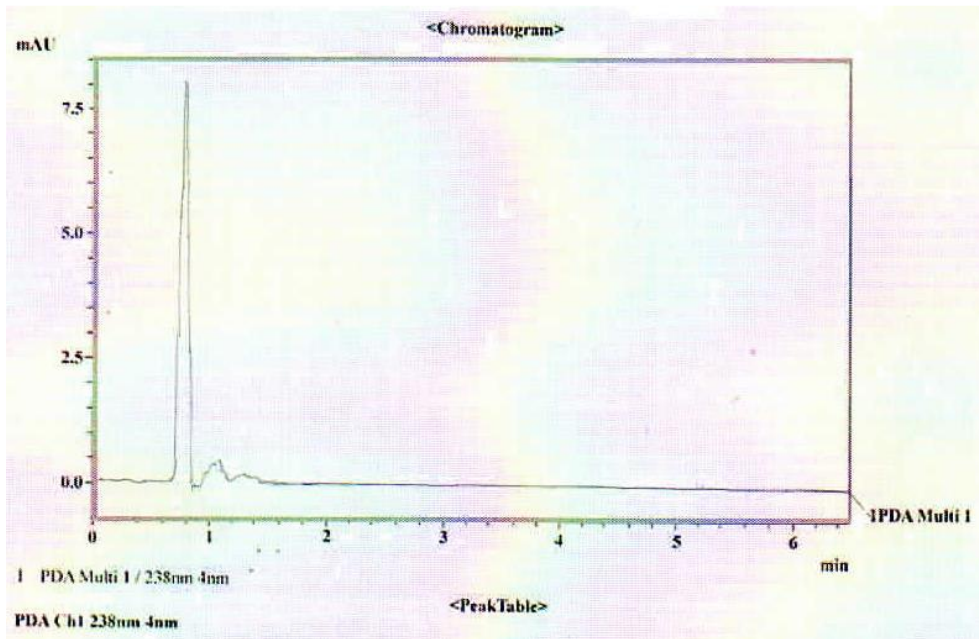
Buffer solutions (with pH of 4.0, & 7.0 or 9.2)

Procedure

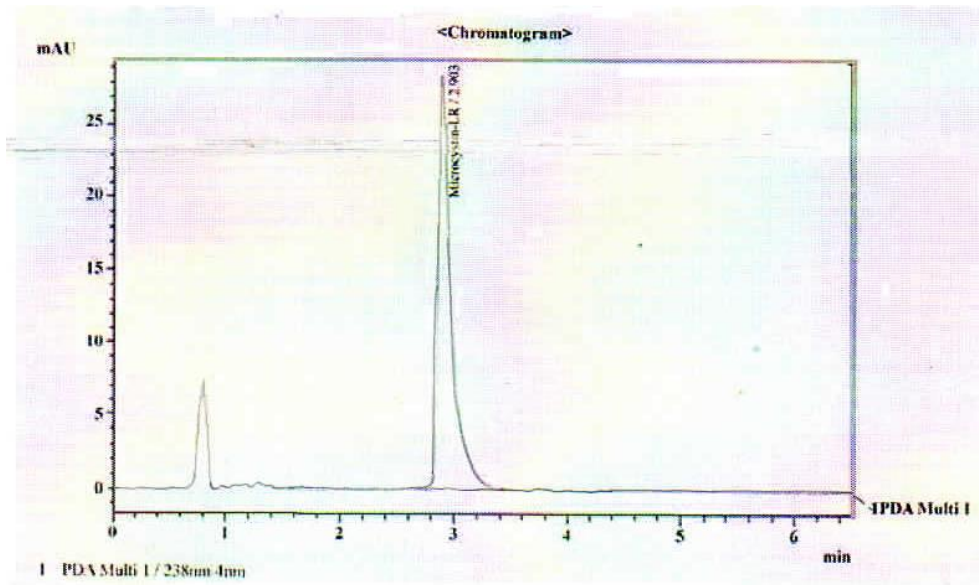
1. The pH meter / ion analyser was connected to the mains
2. The electrodes was connected to the meter.
3. The meter was set to pH mode using freshly prepared buffer solutions. The meter was standardised by immersing the electrode into the appropriate buffer (starting with the highest) and the reading observed and adjusted to that of the pH of the buffer
4. The electrode was rinsed and immersed into the second buffer solution and the reading adjusted as (3) above.
5. (3) and (4) above was repeated until there was no further adjustment for the second buffer solution, i.e., when the electrodes are immersed on the second buffer solution the reading observed was the pH of that buffer.
6. After standardising the meter, the electrode(s) was rinsed and immerse in the sample of the unknown pH, the reading observed was the pH of that sample.

APPENDIX VI Chromatograms of cyanotoxins level in selected water sources

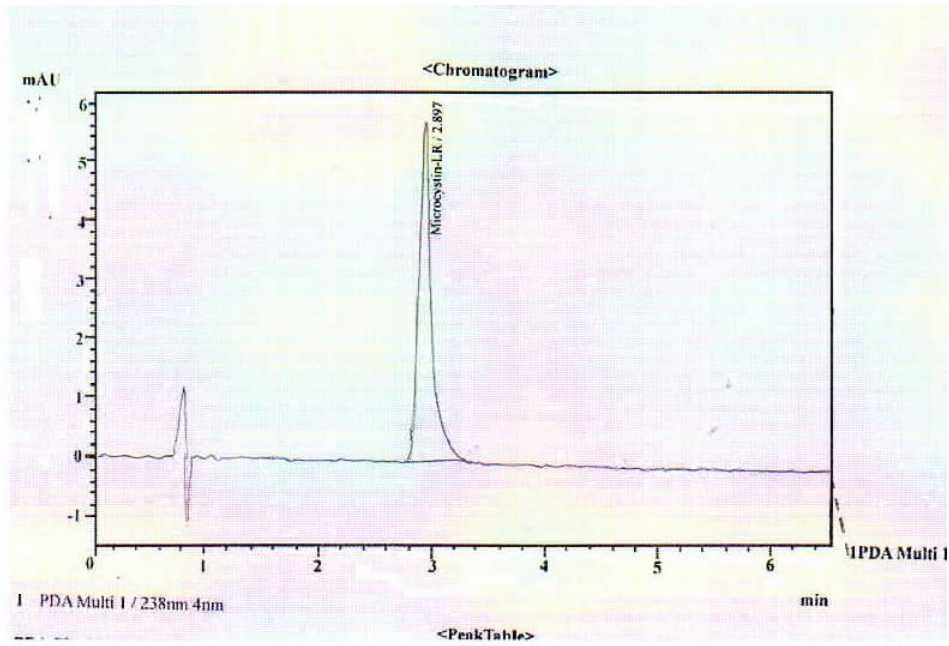
Blank



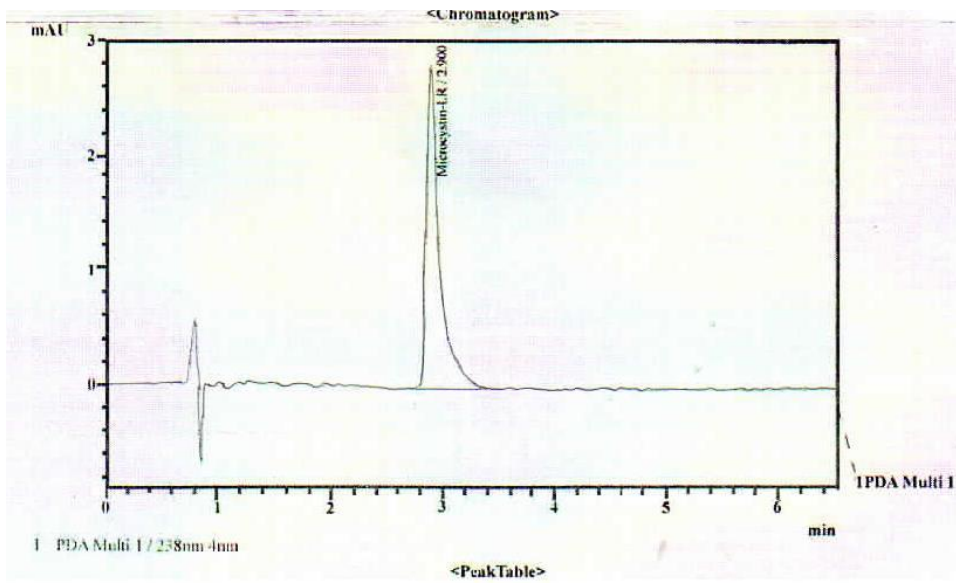
Standard



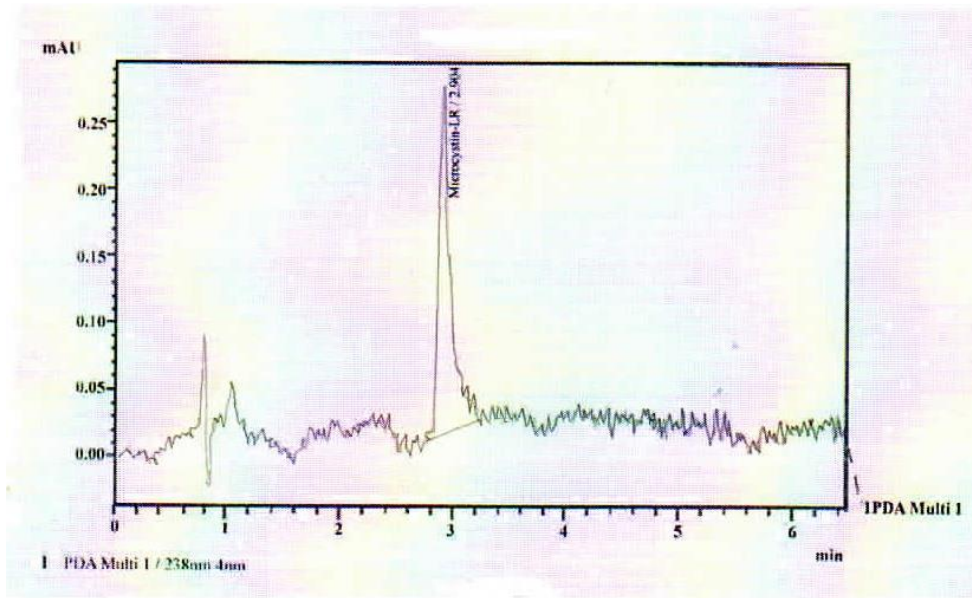
Kijji water source



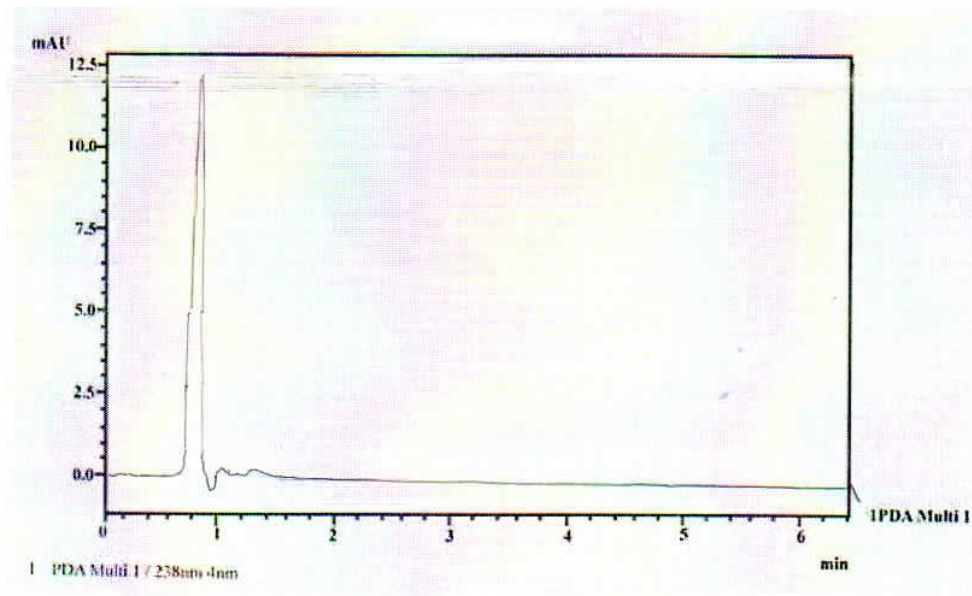
Kaiboche water source



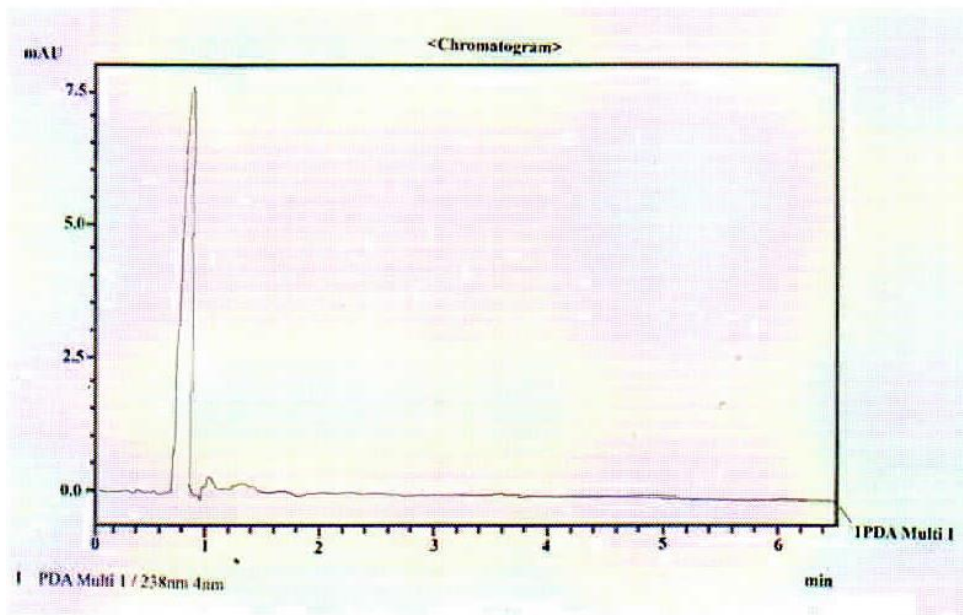
Gituma water source



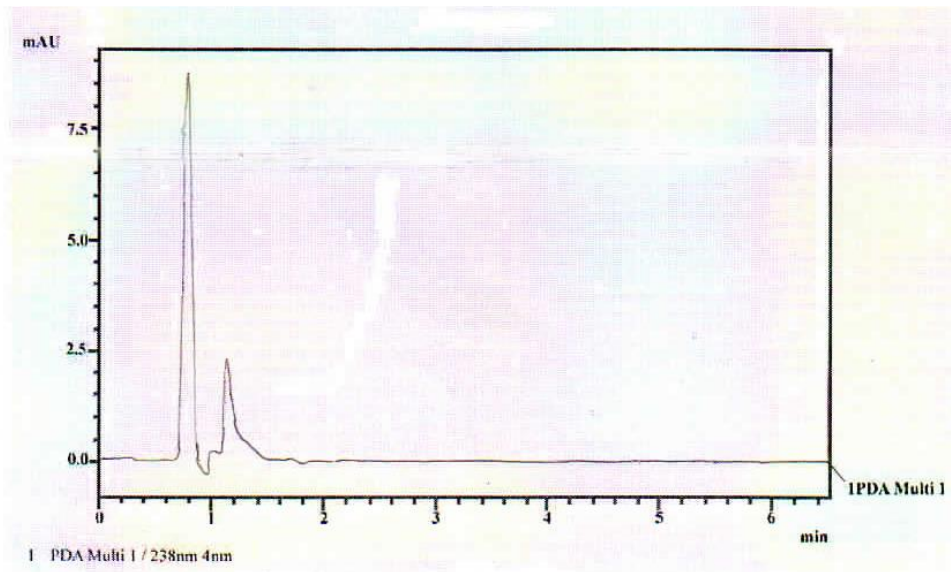
Kaguuma water source



Ntugi water source




Marimanti tap water



APPENDIX VII Physicochemical characteristics of selected water sources

Source	Total Nitrogen	Total Phosphate	pH	Turbidity	Temperature
Kiyii	7.01	2.16	9.3	21.2	27.6
	7.02	2.16	9.31	21.2	27.59
	7.01	2.16	9.3	21.23	27.59
AV	7.01333	2.16	9.30333	21.21	27.5933
Kaiboche	5.44	1.85	9.8	20.8	26.3
	5.45	1.85	9.79	20.8	26.3
	5.45	1.85	9.78	20.8	26.3
AV	5.44667	1.85	9.79	20.8	26.3
Gituma	6.11	1.88	8.6	20	26.9
	6.11	1.89	8.62	20	26.6
	6.12	1.88	8.63	20	26.6
AV	6.11333	1.88333	8.61667	20	26.7
Kaaguma	3.2	1.02	8.3	11	21.7
	3.2	1.01	8.3	11	21.7
	3.2	1.02	8.3	11	21.7
AV	3.2	1.01667	8.3	11	21.7
Ntugi	2.14	1.42	8.7	13.4	19.3
	2.14	1.42	8.69	13.4	19.3
	2.14	1.42	8.69	13.4	19.3
AV	2.14	1.42	8.69333	13.4	19.3
Marimanti	2.47	0.98	7.8	6.4	18
	2.47	0.98	7.78	6.4	18
	2.47	0.98	7.78	6.4	18
AV	2.47	0.98	7.78667	6.4	18

APPENDIX VIII Ethical approval by Kenyatta University


KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
secretariat.kuerc@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Tel: 8710901/12

Our Ref: KU/R/COMM/51/822 Date: 9th November, 2016

Dickson W. Wakhungu
Kenyatta University
P.O. Box 43844 -00100
NAIROBI

Dear Dickson,

APPLICATION NUMBER **PKU/569/1658** – “EVALUATION OF CYANOTOXIN LEVELS IN MAN-MADE WATER RESERVOIRS AND ASSOCIATED WATER RELATED DISEASE BURDEN IN THARAKA NITHI COUNTY, KENYA” – VERSION 2

1. IDENTIFICATION OF PROTOCOL
The application before the committee is with a research topic “Evaluation Of Cyanotoxin Levels in Man-Made Water Reservoirs and Associated Water Related Disease Burden in Tharaka Nithi County, Kenya” - Version 2 received on 17th October, 2016 and discussed on 8th November, 2016.

2. APPLICANT
Dickson W. Wakhungu

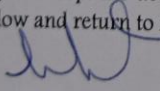
3. SITE
Tharaka Nith County, Kenya

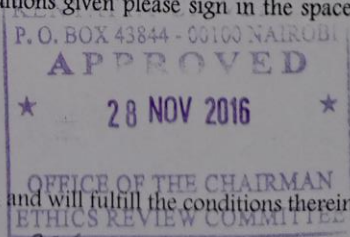
4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 9th November, 2016.

5. ADVICE/CONDITIONS

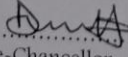
- i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
- ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
- iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
- iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.


DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE



I Dickson Wakhungu accept the advice given and will fulfill the conditions therein.


Signature:  Dated this day of 28.11.2016 2016.

cc. Vice-Chancellor
DVC-Research Innovation and Outreach

Appendix X NACOSTI permit

THIS IS TO CERTIFY THAT: **Permit No : NACOSTI/P/17/97036/15373**
MR. DICKSON WEKESA WAKHUNGU **Date Of Issue : 13th February, 2017**
of KENYATTA UNIVERSITY, 12-60215 **Fee Received :Ksh 1000**
Marimanti, has been permitted to
conduct research in Tharaka-Nithi
County

on the topic: EVALUATION OF
CYANOTOXIN LEVELS IN MAN-MADE
WATER RESERVOIRS AND ASSOCIATED
WATER RELATED DISEASE BURDEN IN
THARAKA-NITHI COUNTY, KENYA,
for the period ending:
11th February, 2018




[Signature]
Director General
National Commission for Science,
Technology & Innovation


[Signature]
Applicant's
Signature

CONDITIONS

- 1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.**
- 2. Government Officer will not be interviewed without prior appointment.**
- 3. No questionnaire will be used unless it has been approved.**
- 4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.**
- 5. You are required to submit at least two(2) hard copies and one (1) soft copy of your final report.**
- 6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice**



REPUBLIC OF KENYA



National Commission for Science,
Technology and Innovation

RESEACH CLEARANCE
PERMIT

Serial No.A 12779

CONDITIONS: see back page